

**E**pidemiological studies have repeatedly shown that populations whose diets include plenty of fruits and vegetables have lower rates of cancer, heart disease, and other “ailments of aging.”

That’s why fruits and vegetables are high on the list of recommended foods in the *Dietary Guidelines for Americans*.

Scientists are keenly interested in learning which substances make fruits and vegetables so healthful. During the last decade, their curiosity has introduced us to a whole new language of phytonutrients, the beneficial compounds in plant foods. At the forefront of this inquiry are chemists in ARS’s Food Composition Laboratory at Beltsville, Maryland. Their collaboration with a sister laboratory has resulted in two phytonutrient databases.

In 1998, ARS’ Nutrient Data Laboratory, the group responsible for maintaining the national nutrient

databank, launched a database giving levels of various carotenoids—such as beta carotene or lycopene—in plant-based foods, thanks to the analytical expertise of researchers in ARS’ Food

measure the promising flavonoids in all plant foods,” says Beecher. Until now, he says, chemists have had to tailor their flavonoid analyses to different types of foods. He expects the new system will

be adopted by university scientists and commercial laboratories.

Western diets provide from several milligrams to a gram of flavonoids each day. The list of their health-giving properties is lengthy and growing. Various flavonoids

have been shown to prevent oxidation, chelate (bind) metals, stimulate the immune system and also reduce an allergic response, prevent formation of carcinogens, impede cancer cell growth, and protect against bacteria and viruses.

### Getting Them All With One Shot

Merken explains that foods contain more than 50 flavonoids, and they fit into 5 major subclasses: anthocyanidins, catechins, flavanones, flavones, and flavonols (see list). He says several

## Finessing the Flavonoids

Composition Laboratory. In 1999, a database of the isoflavones—like genistein and daidzein—in soy foods went online. You can view these databases at: <http://www.nal.usda.gov/fnic/foodcomp/Data/index.html>.

Now, the two laboratories are readying a database of flavonoids—the largest class of phytonutrients—for its electronic debut. ARS chemists Howard M. Merken and Gary R. Beecher developed what they hope will be “a universal system to

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While monitoring the process of raspberry, blueberry, and strawberry flavonoid extractions, technician Casandra Merken checks the solvent level of the blueberry mixture.

### Some Food Sources of Flavonoids

#### **Anthocyanidins** (cyanidin and delphinidin)

berries, grapes, fruit skins, and true fruit juices

#### **Catechins** (catechin and epicatechin)

true teas (not herbal teas)

#### **Flavanones** (hesperetin and naringenin)

citrus

#### **Flavones** (apigenin and luteolin)

grains and herbs

#### **Flavonols** (myricetin and quercetin)

fruits, onions, and botanicals

methods exist for identifying and measuring one or two, maybe even three, of the subclasses. But mixed diets contain flavonoids from all five subclasses. Most of the food flavonoids have glucose or some other sugar attached.

The system Merken developed with Beecher finds and separates the 18 most common food flavonoids, representing all 5 subclasses. The trick in making one system work for all the subclasses, says Merken, was to remove the attached sugars while the flavonoids are being removed from the foods. Most of the current methods measure the flavonoids with their sugars. But commercial standards—pure compounds of known quantity—aren't available for several of the flavonoid glycosides, as the sugar-coated flavonoids are called. The standards are necessary to calibrate the system and ensure accuracy.

Merken says a big hurdle in ensuring accuracy was to account for the progressive loss of some of the flavonoids in the boiling acid needed to extract them from the food. That's especially true for the anthocyanidins—the red and blue pigments that give berries, grapes, and other fruit skins their visual appeal. But it also happens to flavonols—the best known being quercetin, which is abundant in onions.

Recognizing a pattern in the way these compounds degraded in the boiling acid, Merken turned to a textbook he had used while teaching freshman chemistry.

"It was pseudo first-order kinetics," he says, "so I could use the same type of math that is used to measure the rate of radioactive decay—the math used in carbon dating.

"We don't lose catechins and flavanones during extraction because the solvents we use are much less destructive," he says. "And the flavones have proved very stable." Except for differences in the extraction method among the flavonoid subclasses, the chromatography is the same. "We use the same

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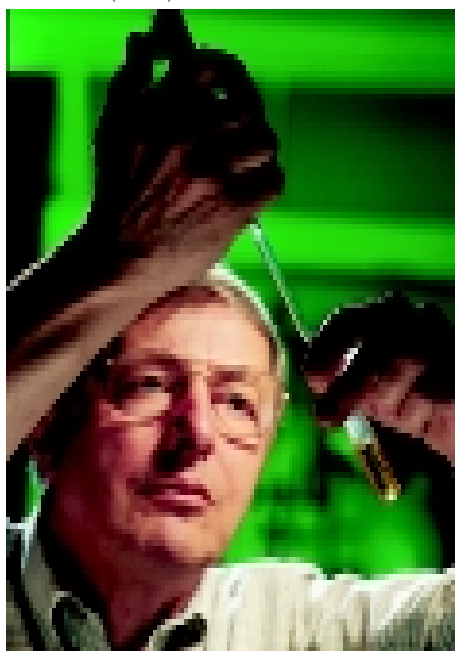


**Chemist Howard Merken analyzes blueberry extract for flavonoids on a high-performance liquid chromatograph.**

high-performance liquid chromatography system to measure all of them," says Merken.

After the system is fine-tuned, Beecher says, it will be able to analyze several foods in one day. He and Merken are using it to provide many of the values in the new flavonoid database. They are analyzing 50 commonly eaten fruits, vegetables, nuts, and other foods sampled by the Nutrient Data Laboratory. The project is partially funded by the National Heart, Lung and Blood Institute

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**Chemist Gary Beecher prepares a raspberry sample for HPLC analysis.**

and the Produce for Better Health Foundation, which sponsors the 5-A-Day program for the fruit and vegetable industry, urging us to eat at least five fruits and vegetables daily.

The foods have been selected from grocery chains around the country by use of a statistically based sampling plan to ensure that they accurately represent the U.S. food supply, says Joanne M. Holden, who heads the Nutrient Data Laboratory. Meanwhile, her group is gathering and evaluating flavonoid data that has already been published in the scientific literature or supplied by the food industry. Acceptable data will be combined with new data being generated by Merken and Beecher. Holden expects the flavonoid database to be available in late 2001.

Beecher also wants the flavonoid data ready for an expert panel being sponsored by the National Academy of Sciences' Food and Nutrition Board. The panel will look at the body of research on some of the emerging phytonutrients to assess the importance of these components to health.

"Epidemiologists need data on all of these food components to draw associations between intake and health status," he notes.—By **Judy McBride, ARS.**

*This research is part of Human Nutrition, an ARS National Program (#107) described on the World Wide Web at <http://www.nps.ars.usda.gov>.*

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